

In re Application of YANG  
Confirmation No: 9213  
Application No. 10/640,989  
Examiner: Afremova, Vera

### **AMENDMENTS TO THE CLAIMS**

This listing of claims will replace all prior versions, and listings, of claims in the application:

#### **Listing of Claims:**

1. (Currently Amended). An insulin-producing cell isolated from an *in vitro* culture of bone marrow cells obtained from a human subject.

2. (Withdrawn) A cell that expresses detectable levels of glucagon, insulin, and mRNAs encoding insulin, Pdx-1, and NeuroD, the cell being isolated from a culture of human bone marrow cells prepared by a method comprising the steps of:

(a) obtaining human bone marrow mononuclear cells from a human subject; and

(b) culturing the obtained human bone marrow mononuclear cells under conditions that cause the cells to express detectable levels of glucagon, insulin, and mRNAs encoding insulin, Pdx-1, and NeuroD.

3. (Withdrawn) The cell of claim 2, wherein the step (b) of culturing the human bone marrow mononuclear cells under conditions that cause the cells to express detectable levels of glucagon, insulin, and mRNAs encoding insulin, Pdx-1, and NeuroD comprises:

(i) first, culturing the human bone marrow mononuclear cells in a tissue culture container for about 24 to 48 hours to obtain cells that adhere to the container (adherent cells), and continuing to culture the adherent cells until they become morphologically homogenous; and

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(ii) second, culturing the morphologically homogeneous cells for at least about 60 days in a medium comprising about 9 to 30 mM glucose at least until the cells express detectable levels of glucagon, insulin, and mRNAs encoding insulin, Pdx-1, and NeuroD.

4. (Withdrawn) The method of claim 3, wherein the medium comprises about 23 mM glucose.

5. (Withdrawn) The cell of claim 3, wherein step (ii) further comprises culturing the cells for at least about 60 days in a medium comprising at least one growth factor selected from the group consisting of: FGF, EGF, and HGF.

6. (Withdrawn) The cell of claim 3, wherein the step (b) of culturing the human bone marrow mononuclear cells under conditions that cause the cultured cells to express detectable levels of glucagon, insulin, and mRNAs encoding insulin, Pdx-1, and NeuroD further comprises:

(iii) third, culturing the cells for about 5 to 7 days in a medium comprising less than about 7.5 mM glucose and at least one agent selected from the group consisting of nicotinamide and exendin 4.

7. (Withdrawn). The cell of claim 2, wherein the cell secretes insulin when placed in a medium comprising about 23 mM glucose.

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8. (Currently Amended). The cell of claim 1, wherein the cell is ~~comprised~~ cultured in a liquid.
9. (Original) The cell of claim 8, wherein the liquid is a tissue culture medium.
10. (Withdrawn) The cell of claim 2, wherein the cell is comprised in a liquid.
11. (Withdrawn) The cell of claim 10, wherein the liquid is a tissue culture medium.
12. (Currently Amended). The cell of claim 1, wherein the insulin producing cell is at a temperature below 0° C.
13. (Currently Amended). The cell of claim 12, wherein the insulin producing cell is housed in a container in liquid nitrogen.
14. (Withdrawn) The cell of claim 2, wherein the cell is at a temperature below 0° C.
15. (Withdrawn) The cell of claim 14, wherein the cell is housed in a container in liquid nitrogen.
16. (Withdrawn) The cell of claim 1, wherein the cell has been introduced into a host animal subject.

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17. (Withdrawn) The cell of claim 2, wherein the cell has been introduced into a host animal subject.

18. (Withdrawn) The cell of claim 16, wherein the cell host animal subject is the human subject from which the human bone marrow cells were obtained.

19. (Withdrawn) The cell of claim 17, wherein the cell host animal subject is the human subject from which the human bone marrow mononuclear cells were obtained.

20. (Withdrawn) A method for making cells that express detectable levels of glucagon, insulin, and mRNAs encoding insulin, Pdx-1, and NeuroD, the method comprising the steps of:

- (a) isolating human bone marrow mononuclear cells from a human subject; and
- (b) culturing the isolated human bone marrow cells under conditions that cause the cultured cells to express detectable levels of glucagon, insulin, and mRNAs encoding insulin, Pdx-1, and NeuroD.

21. (Withdrawn) The method of claim 20, wherein the step (b) of culturing the isolated human bone marrow cells under conditions that cause the cultured cells to express detectable levels of glucagon, insulin, and mRNAs encoding insulin, Pdx-1, and NeuroD comprises:

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(i) first, culturing the human bone marrow mononuclear cells in a tissue culture container for about 24 to 48 hours to obtain cells that adhere to the container (adherent cells), and continuing to culture the adherent cells until they become morphologically homogenous; and

(ii) second, culturing the morphologically homogeneous cells for at least about 60 days in a medium comprising about 9 to 30 mM glucose at least until the cells express detectable levels of glucagon, insulin, and mRNAs encoding insulin, Pdx-1, and NeuroD.

22. (Withdrawn) The method of claim 21, wherein step (ii) further comprises culturing the cells for at least about 60 days in a medium comprising at least one growth factor selected from the group consisting of: FGF, EGF, and HGF.

23. (Withdrawn) The method of claim 21, wherein the step (b) of culturing the isolated human bone marrow cells further comprises:

(iii) third, culturing the cells for about 5 to 7 days in a medium comprising less than about 7.5 mM glucose and at least one agent selected from the group consisting of nicotinamide and exendin 4.

24. (Withdrawn) A method of reducing hyperglycemia in an animal subject, the method comprising transplanting into the animal subject an effective number of pancreatic marker-expressing cells differentiated from human bone marrow-derived stem cells by a method comprising the step of culturing the human bone marrow-derived stem cells in a high glucose containing medium.